TITLE OF THE INVENTION

c-Kit kinase inhibitor

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a c-Kit kinase inhibitor, a therapeutic agent for a disease caused by the excessive activation of c-Kit kinase comprising c-Kit kinase inhibitor as an active ingredient.

Related Background of the Invention

[0002] Intracellular signal transduction by receptor tyrosine kinase contributes to cell proliferation, differentiation and metabolism; as a result, it is responsible for various diseases including cancers (Kolibaba K. S. et al., B.B.A. 1333, F217-F248, 1997; and Sheijen B. et al. Oncogene 21, 3314-3333, 2002).

[0003] c-Kit kinase, one of receptor tyrosine kinase, binds to SCF (stem cell factor) which is a ligand specific for the kinase. This causes dimerization of the kinase itself and the subsequent activation of the kinase activity. Consequently, a variety of substrates of c-Kit kinase in cells will be phosphorylated (Blume-Jensen P. et al., EMBO J. 10, 4121-4128, 1991; and Lev S. et al., EMBO J., 10, 647-654, 1991).

[0004] The abnormal activation of c-Kit kinase generates a proliferation signal in certain types of cancer cells (their representatives are described below), which is regarded as the cause of cancerization or malignant transformation.

[0005] (1) Acute myelogenous leukemia (AML): The expression of c-Kit kinase was found in a number of patients (60-80%) suffering from acute myelogenous leukemia and the proliferation of blast derived from the patients was stimulated by SCF. Furthermore, in 13 out of 18 patients the activation of c-Kit kinase was observed without SCF stimulation. It was then thought that activating mutations of c-Kit kinase occurred in these patients (Lev S. et al., EMBO J., 10, 647-654, 1991; Wang C et al., Leukemia 3, 699-702, 1989; Kanakura Y. et al., Leuk. Lymph. 10, 35-41, 1993; Ikeda H. et al., Blood, 78, 2962-2968, 1991; and Ikeda H. et al., Exp. Hematol. 21, 1686-1694, 1993).

[0006] (2) Mast cell leukemia: There was a report that activating mutations of c-Kit kinase was found in the cell line of mast cell leukemia a mastocytosis patient had developed (Furitsu T. et al., J. Clin. Invest., 92, 1736-1744, 1993).

[0007] (3) Small cell lung cancer (SCLC): While high level expression of c-Kit kinase was observed in more than 70% of SCLC cell lines, the expression levels

of c-Kit kinase in the cell lines of non-small cell lung cancers were either low or below the detection limit. SCF, a ligand for c-Kit kinase, is also expressed in the cell lines of SCLC. This suggested the possibility that autocrine proliferation was promoted (Hibi K. et al., Oncogene, 6, 2291-2296, 1991; and Sekido Y. et al., Cancer Res., 51, 2416-2419, 1991).

[0008] (4) GIST (gastrointestinal stromal tumors): GIST is defined as a stromal tumor that develops in the GI tract expressing c-Kit kinase. In about a half of GIST, activating mutations of c-Kit kinase was found and it was present at high frequency in GIST with high malignancy. This suggested the possibility of the mutation being a prognosis factor (Lasota J. et al., Am. J. Pathol., 157, 1091-1095, 2000; and Taniguchi M. et al., Cancer Res., 59, 4297-4300, 1999).

[0009] (5) Testicular cancer: In testicular cancer, carcinoma in situ (CIS), which is regarded as a precancerous lesion, progresses to form tumors which are referred to as "seminoma" and "non-seminoma." High-level expression of c-Kit kinase in CIS and seminoma was reported (Stromeyer T. et al., Cancer Res., 51, 1811-1816, 1991). In recent years there has been a report on the expression of c-Kit kinase that underwent an activating mutation in seminoma (Tian Q. et al., Am. J. Pathol., 154, 1643-1647, 1999).

[0010] (6) Ovarian cancer: There has been reported as follows. In normal ovarian epithelia, SCF was expressed but the expression of c-Kit kinase was not observed. However, c-Kit kinase and SCF were both expressed in benign ovarian tumor at an early stage of cancerization; oppositely, the expression of c-Kit kinase was lowered in malignant ovarian tumor. These results suggested that c-Kit kinase played an important role in the development of ovarian cancer (Tonary A. T., Int. J. Cancer, 89, 242-250, 2000).

[0011] (7) Breast cancer: There was a report that the expression of c-Kit kinase was lowered in breast cancer as compared to the surrounding normal tissues (Natali P. et al., Int. J. Cancer, 52, 713-717, 1992). However, in later studies the expression of c-Kit kinase, which had not been detected in normal tissue, was observed in breast cancer and SCF expression was also detected. These suggested that the autocrine stimulation promoted proliferation (Hines S. J. et al., Cell Growth & Differentiation, 6, 769-779, 1995).

[0012] (8) Brain cancer: There has been reported as follows: c-Kit kinase expression was observed in the cell line and tissue of glioblastoma that had the highest level of malignancy among brain cancers; and in the glioblastoma cell line expressing c-Kit kinase SCF stimulation promoted growth (Berdel W. E. et al.,

Cancer Res., 52, 3498-3502, 1992).

[0013] (9) Neuroblastoma: There has been reported as follows. SCF and c-Kit kinase were coexpressed in many cases of the cell lines and the tissue specimens of neuroblastoma which was well known as the cancer that developed in infants. Anti-c-Kit kinase antibody suppressed the growth of the cell line of neuroblastoma, and thus, growth was promoted by an autocrine mechanism (Cohen P. S., Blood, 84, 3465-3472, 1994).

[0014] (10) Colorectal cancer: Coexpression of c-Kit kinase and its ligand, SCF, was observed in a colorectal cancer tissue, whereas the expression of neither one was observed in a normal mucosal tissue. SCF stimulation promoted proliferation of the colorectal cancer cell line (Bellone G. et al., J. Cell. Physiol., 172,1-11, 1997).

[0015] It was reported that the activation of c-Kit kinase by SCF stimulation was essential to proliferation and differentiation of mast cells (Hamel et al., J. Neuro-Onc., 35, 327-333, 1997; and Kitamura et al., Int. Arch. Aller. Immunol., 107, 54-56, 1995). It has, therefore, been thought that the excessive activation of c-Kit kinase is responsible for immunological abnormalities (such as mastocytosis, asthma and chronic rhinitis) which are caused by the excessive mast cells.

[0016] (1) Mastocytosis: Mastocytosis is a general term for the pathology of various conditions characterized by the excessive growth of mast cells (Metcalf, J. Invest. Derm. 93, 2S-4S, 1991; and Golkar et al., Lancet, 349, 1379-1385, 1997). The following have been reported on mastocytosis patients: 1) the excessive expression of c-Kit kinase (Nagata et al., Mastocytosis Leuk., 12, 175-181, 1998); 2) an increase in the amount of soluble SCF (Longley et al., New Engl. J. Med., 328, 1302-1307, 1993); and 3) activating mutations of c-Kit kinase (Nagata et al., Mastocytosis Leuk., 12, 175-181, 1998; and Longley et al., Nat. Gen., 12, 312-314, 1996). These are believed to excessively activate c-Kit kinase and thus to cause mastocytosis.

[0017] (2) Allergy and asthma: Mast cells and eosinophils are important cells in the development of inflammation, allergy, asthma and the like (Thomas et al., Gen. Pharmacol., 27, 593-597, 1996; and Metcalf et al., Physiol. Rev., 77, 1033-1079, 1997). This is suggested by the report that corticosteroids which are currently believed to be most effective against inflammations involving chronic rhinitis or allergy decrease the numbers of circuiting and invading mast cells and eosinophils (Naclerio et al., JAMA, 278, 1842-1848, 1997; and Meltzer, Aller., 52, 33-40, 1997). The activation of c-Kit kinase resulting from SCF

stimulation was not only essential to differentiation, survival and proliferation of mast cells, but also promoted the induction of various factors from the mast cells. These factors fulfilled an important function in differentiation, survival and invasiveness of the eosinophils (Okayama et al., Int. Arch. Aller. Immunol., 114, 75-77, 1997; Okayama et al., Eur. J. Immunol., 28, 708-715, 1998; Metcalf et al., Proc. Natl. Acad. Sci., 95, 6408-6421, 1998; Kay et al., Int. Arch. Aller. Immunol., 113, 196-199, 1997; Hogaboam et al., J. Immunol. 160, 6166-6171, 1998; and Luckas et al., J. Immunol. 156, 3945-3951, 1996). It has, therefore, been thought that the inhibition of c-Kit kinase can suppress the activated mast cells and eosinophils in the patients suffering from asthma or allergy.

[0018] As stated above, c-Kit kinase is believed to be closely involved in the development or the malignant transformation of some types of cancers as well as in the diseases for which excessive mast cells are regarded as the cause. Inhibitors of c-Kit kinase have been considered useful as therapeutic agents for those diseases.

SUMMARY OF THE INVENTION

[0019] The problem to be solved by the invention is to discover a novel compounds exhibiting c-Kit kinase inhibitory activity and to develop a therapeutic

agent for diseases caused by c-Kit kinase.

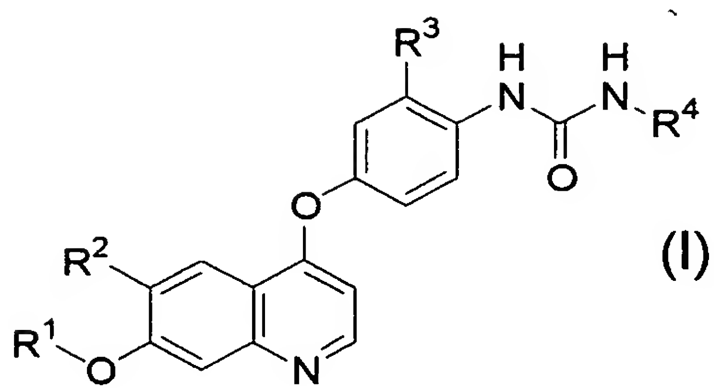
[0020] Compounds having an indoline skeleton were reported as those showing c-Kit kinase inhibitory action (WO 01/45689). There was also a report concerning the inhibitory action on c-Kit kinase by the compounds having a quinazoline skeleton (WO 01/47890). An analogue (KRN633) was also reported to possess c-Kit kinase inhibitory action (Kazuo Kubo et al., 22nd Symposium on Medicinal Chemistry, Abstracts, pp. 275-277, 2P-320, 2002). Recently, Gleevec (STI571) was approved in U.S., Europe and Japan as a therapeutic agent for GIST based on c-Kit inhibition (Drugs, 63: 513-22, 2003).

[0021] We have reported that a compound represented by the following general formula (I) inhibits kinase activity of VEGF receptor, and that it also inhibits tube formation of vascular endothelial cells stimulated by VEGF, FGF2 or HGF (WO02/32872). And, we discovered that a compound represented by the following general formula (I) inhibits not only VEGF kinase but also c-Kit kinase, and that it has an inhibitory activity against proliferation of cancer cells expressing c-Kit kinase.

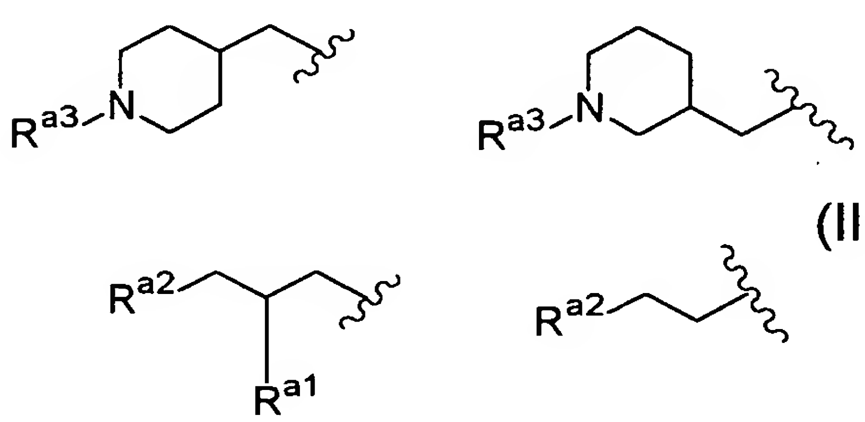
[0022] Specifically, the invention relates to:

<1> A c-Kit kinase inhibitor comprising as an active ingredient, a compound represented by the general

formula (I), a salt thereof or a hydrate of the foregoing:


(I)

(wherein R^1 represents methyl, 2-methoxyethyl or a group represented by the formula (II):


(II)

(wherein R^{a3} represents methyl, cyclopropylmethyl or cyanomethyl; R^{a1} represents hydrogen, fluorine or hydroxyl; and R^{a2} represents 1-pyrrolydinyl, 1-piperidinyl, 4-morpholinyl, dimethylamino or diethylamino);

R^2 represents cyano or $-CONHR^{a4}$ (wherein R^{a4} represents hydrogen, C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkoxy or C_{3-8} cycloalkoxy);

R^3 represents hydrogen, methyl, trifluoromethyl, chlorine or fluorine; and

R^4 represents hydrogen, methyl, ethyl, n-propyl, cyclopropyl, 2-thiazolyl or 4-fluorophenyl).

<2> The c-Kit kinase inhibitor according to <1>, wherein R^1 represents methyl.

<3> The c-Kit kinase inhibitor according to <1>, wherein R^4 represents methyl, ethyl or cyclopropyl.

<4> The c-Kit kinase inhibitor according to <1>, wherein R^3 represents hydrogen, chlorine or fluorine.

<5> The c-Kit kinase inhibitor according to <1>, wherein R^2 represents $-CONHR^{a4}$ (wherein R^{a4} represents hydrogen or methoxy).

<6> The c-Kit kinase inhibitor according to <1>, wherein the compound represented by the general formula (I) is a compound selected from the group consisting of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide, 4-(3-chloro-4-(ethylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide, N6-methoxy-4-(3-chloro-4-(((cyclopropylamino)carbonyl)amino)phenoxy)-7-methoxy-6-quinolinecarboxamide and N6-methoxy-4-(3-chloro-4-(((ethylamino)carbonyl)amino)phenoxy)-7-methoxy-6-quinolinecarboxamide.

<7> An anticancer agent for treating a cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase, comprising as an active ingredient, the c-Kit kinase inhibitor according to <1>.

<8> The anticancer agent according to <7>, wherein the cancer expressing excessive c-Kit kinase or a mutant c-

Kit kinase is acute myelogenous leukemia, mast cell leukemia, a small cell lung cancer, GIST, a testicular cancer, an ovarian cancer, a breast cancer, a brain cancer, neuroblastoma or a colorectal cancer.

<9> The anticancer agent according to <7>, wherein the cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase is acute myelogenous leukemia, a small cell lung cancer or GIST.

<10> The anticancer agent according to <7>, which is applied to a patient for which a cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase is identified.

<11> A therapeutic agent for mastocytosis, allergy or asthma, comprising as an active ingredient, the c-Kit kinase inhibitor according to <1>.

<12> A therapeutic method for a cancer, comprising administering to a patient suffering from a cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase, a pharmacologically effective dose of the c-Kit kinase inhibitor according to <1>.

<13> The method according to <12>, wherein the cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase is acute myelogenous leukemia, mast cell leukemia, a small cell lung cancer, GIST, a testicular cancer, an ovarian cancer, a breast cancer, a brain cancer, neuroblastoma or a colorectal cancer.

<14> The method according to <12>, wherein the cancer expressing excessive c-Kit kinase or a mutant c-Kit kinase is acute myelogenous leukemia, a small cell lung cancer or GIST.

<15> A therapeutic method for a cancer, comprising the steps of:

extracting cancer cells from a patient suffering from a cancer;

confirming that the cancer cells are expressing excessive c-Kit kinase or a mutant c-Kit kinase; and

administering to the patient a pharmacologically effective dose of the c-Kit kinase inhibitor according to <1>.

<16> A therapeutic method for mastocytosis, allergy or asthma, comprising administering to a patient suffering from the disease, a pharmacologically effective dose of the c-Kit kinase inhibitor according to <1>.

<17> A method for inhibiting the c-Kit kinase activity, comprising applying to a cell expressing excessive c-Kit kinase or a mutant c-Kit kinase, a pharmacologically effective dose of the c-Kit kinase inhibitor according to <1>.

[0023] A compound showing a strong c-Kit kinase inhibitory activity has been discovered, a therapeutic agent for suppressing cancerization and malignant transformation of certain kind of cancer, or a

therapeutic agent for diseases considered to be caused by c-kit kinase, such as mastocytosis, allergy or asthma can be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Fig. 1 is a graph showing the results of immunoblot of phosphorylated c-Kit kinase by SCF stimulation.

[0025] Fig. 2 is a graph showing the relationship between the numbers of days elapsed after transplantation and tumor volume when H-526 was transplanted to a nude mouse.

[0026] Fig.3 is a graph showing the result of immunoblot of phosphorylated c-Kit kinase, c-Kit kinase and β-actin when H-526 was transplanted to a nude mouse.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0027] The embodiments of the present invention will be explained below.

[0028] Several of the structural formulas given for compounds throughout the present specification will represent a specific isomer for convenience, but the invention is not limited to such specific isomers and encompasses all isomers and isomer mixtures, including geometric isomers, asymmetric carbon-derived optical isomers, stereoisomers and tautomers, implied by the structures of the compounds. Moreover, the compounds of the invention also include those that have been

metabolized in the body by oxidation, reduction, hydrolysis, conjugation or the like, and still exhibit the desired activity, while the invention further encompasses all compounds which undergo metabolism such as oxidation, reduction, hydrolysis, etc. in the body to produce the compounds of the invention. Solvates, including those with water, are also encompassed by the invention.

[0029] The term "C_{1-6} alkyl" as used throughout the present specification refers to linear or branched alkyl of 1 to 6 carbons, and as specific examples there may be mentioned methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, i-pentyl, sec-pentyl, t-pentyl, neopentyl, 1-methylbutyl, 2-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, n-hexyl, i-hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl and 1-ethyl-2-methylpropyl, preferably methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, i-pentyl, sec-pentyl, t-pentyl, neopentyl, 1-methylbutyl, 2-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, n-hexyl and i-hexyl, more preferably methyl, ethyl, n-propyl, i-propyl, n-

butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, i-pentyl, sec-pentyl, t-pentyl, neopentyl, 1-methylbutyl, 2-methylbutyl, 1,1-dimethylpropyl and 1,2-dimethylpropyl, even more preferably methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl and t-butyl, and most preferably methyl, ethyl, n-propyl and i-propyl.

[0030] The term "C_{3-8} cycloalkyl" as used throughout the present specification refers to cyclic alkyl of 3 to 8 carbons, and as specific examples there may be mentioned cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, with cyclopropyl being preferred.

[0031] The term "C_{1-6} alkoxy" as used throughout the present specification refers to a substituent wherein the aforementioned "C_{1-6} alkyl" is bonded to oxygen, and as specific examples there may be mentioned methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, sec-butoxy, t-butoxy, n-pentyloxy, i-pentyloxy, sec-pentyloxy, t-pentyloxy, neopentyloxy, 1-methylbutoxy, 2-methylbutoxy, 1,1-dimethylpropoxy, 1,2-dimethylpropoxy, n-hexyloxy, i-hexyloxy, 1-methylpentyloxy, 2-methylpentyloxy, 3-methylpentyloxy, 1,1-dimethylbutoxy, 1,2-dimethylbutoxy, 2,2-dimethylbutoxy, 1,3-dimethylbutoxy, 2,3-dimethylbutoxy, 3,3-dimethylbutoxy, 1-ethylbutoxy, 2-ethylbutoxy, 1,1,2-trimethylpropoxy, 1,2,2-trimethylpropoxy, 1-ethyl-1-methylpropoxy and 1-ethyl-2-methylpropoxy,

preferably methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, sec-butoxy, t-butoxy, n-pentyloxy, i-pentyloxy, sec-pentyloxy, t-pentyloxy, neopentyloxy, 1-methylbutoxy, 2-methylbutoxy, 1,1-dimethylpropoxy, 1,2-dimethylpropoxy, n-hexyloxy and i-hexyloxy, more preferably methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, sec-butoxy, t-butoxy, n-pentyloxy, i-pentyloxy, sec-pentyloxy, t-pentyloxy, neopentyloxy, 1-methylbutoxy, 2-methylbutoxy, 1,1-dimethylpropoxy and 1,2-dimethylpropoxy, even more preferably methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, sec-butoxy and t-butoxy, and most preferably methoxy, ethoxy, n-propoxy and i-propoxy.

[0032] The term "C_{3-8} cycloalkoxy" as used throughout the present specification refers to cyclic alkoxy of 3 to 8 carbons, and as specific examples there may be mentioned cyclopropoxy, cyclobutoxy, cyclopentyloxy and cyclohexyloxy, with cyclopropoxy being preferred.

[0033] A compound represented by the general formula (I) can be produced by the method described in WO02/32872.

[0034] Throughout the present specification, the term "pharmacologically acceptable salt" is not particularly restrictive on the type of salt, and as examples of such salts there may be mentioned inorganic

acid addition salts such as hydrochloride, sulfate, carbonate, bicarbonate, hydrobromide and hydroiodide; organic carboxylic acid addition salts such as acetate, maleate, lactate, tartarate and trifluoroacetate; organic sulfonic acid addition salts such as methanesulfonate, hydroxymethanesulfonate, hydroxyethanesulfonate, benzenesulfonate, toluenesulfonate and taurine salts; amine addition salts such as trimethylamine salts, triethylamine salts, pyridine salts, procaine salts, picoline salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, N-methylglucamine salts, diethanolamine salts, triethanolamine salts, tris(hydroxymethylamino)methane salts and phenethylbenzylamine salts; and amino acid addition salts such as arginine salts, lysine salts, serine salts, glycine salts, aspartate and glutamate.

[0035] The dosage of a medicine according to the invention will differ depending on the severity of symptoms, patient age, gender and weight, administration form and type of disease, but administration may usually be from 100 μg to 10 g per day for adults, either at once or in divided doses.

[0036] There are no particular restrictions on the form of administration of a medicine according to the invention, and it may usually be administered orally or parenterally by conventional methods.

[0037] Common excipients, binders, glossy agents, coloring agents, taste correctors and the like, and if necessary stabilizers, emulsifiers, absorption promoters, surfactants and the like, may also be used for formulation, with inclusion of components ordinarily used as starting materials for formulation of pharmaceutical preparations by common methods.

[0038] Examples of such components which may be used include animal and vegetable oils (soybean oil, beef tallow, synthetic glycerides, etc.), hydrocarbons (liquid paraffin, squalane, solid paraffin, etc.), ester oils (octyldodecyl myristate, isopropyl myristate, etc.), higher alcohols (cetostearyl alcohol, behenyl alcohol, etc.), silicone resins, silicone oils, surfactants (polyoxyethylene fatty acid esters, sorbitan fatty acid esters, glycerin fatty acid esters, polyoxyethylenesorbitan fatty acid esters, polyoxyethylene hydrogenated castor oil, polyoxyethylenepolyoxypropylene block copolymer, etc.), water-soluble polymers (hydroxyethyl cellulose, polyacrylic acid, carboxyvinyl polymer, polyethyleneglycol, polyvinylpyrrolidone, methyl cellulose, etc.), alcohols (ethanol, isopropanol, etc.), polyhydric alcohols (glycerin, propyleneglycol, dipropyleneglycol, sorbitol, etc.), sugars (glucose, sucrose, etc.), inorganic powders (silicic anhydride,

aluminium magnesium silicate, aluminium silicate, etc.), purified water and the like. For pH adjustment there may be used inorganic acids (hydrochloric acid, phosphoric acid, etc.), alkali metal salts of inorganic acids (sodium phosphate, etc.), inorganic bases (sodium hydroxide, etc.), organic acids (lower fatty acids, citric acid, lactic acid, etc.), alkali metal salts of organic acids (sodium citrate, sodium lactate, etc.), and organic bases (arginine, ethanolamine, etc.). If necessary, preservatives, antioxidants and the like may also be added.

[EXAMPLES]

[0039] The present invention will be explained through the following examples, but these examples are in no way limitative on the invention.

[0040] [Example 1] Effect on cell proliferation stimulated by SCF

[0041] Compounds 1, 2, 3 and 4 were tested for their effects on the proliferation of the small cell lung cancer cell line H-526 expressing c-Kit kinase (purchased from ATCC: CRL-5811).

Compound 1: 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide

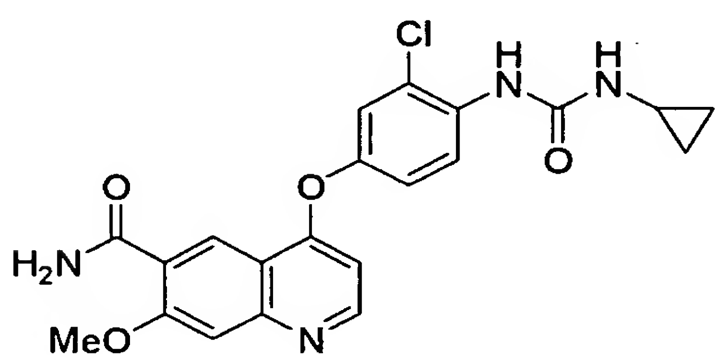
Compound 2: 4-(3-Chloro-4-(ethylaminocarbonyl)aminophenoxy)-7-methoxy-6-

quinolinecarboxamide

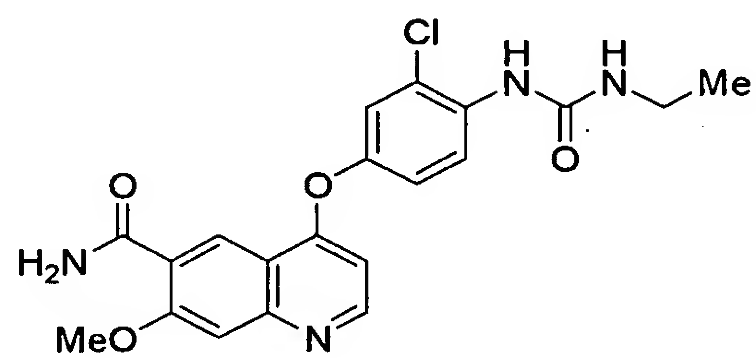
Compound 3: N6-Methoxy-4-(3-chloro-4-(((cyclopropylamino)carbonyl)amino)phenoxy)-7-methoxy-6-quinolinecarboxamide

Compound 4: N6-Methoxy-4-(3-chloro-4-(((ethylamino)carbonyl)amino)phenoxy)-7-methoxy-6-quinolinecarboxamide

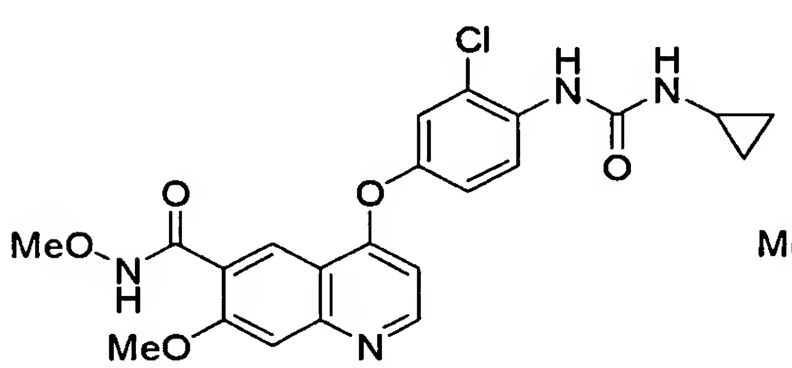
[0042] The structures of Compound 1 to 4 are shown below.



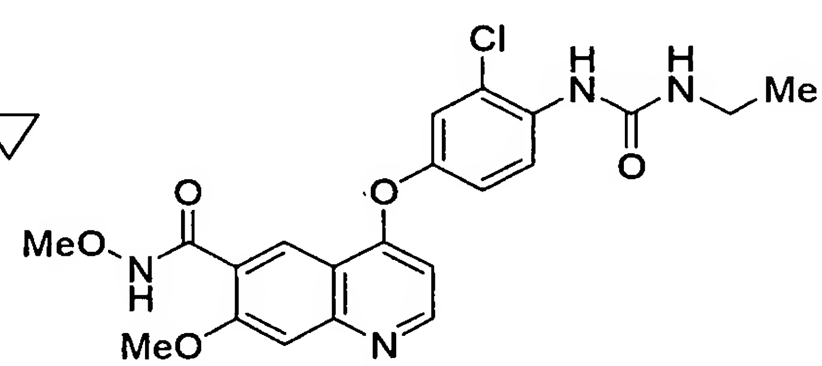
Compound 1



Compound 2



Compound 3



Compound 4

[0043] Compound 1 was prepared by the method described in Example 368 of WO02/32872. Compound 2 was prepared by the method described in Example 583 of WO02/32872. Compound 3 was prepared by the method described in Example 417 of WO02/32872. Compound 4 was prepared by the method described in Example 702 of

WO02/32872.

[0044] H-526 cells were cultured in a 5% CO_2 incubator (37 °c) using an RPMI1640 medium (Nissui Pharmaceutical Co., Ltd.) containing 10% FCS (purchased from Cell Culture Technologies). After culturing, H-526 cells were washed with PBS three times and were suspended in an RPMI1640 medium containing 0.1% BSA (Sigma Corporation) (hereinafter abbreviated as "BSA-RPMI1640") at 1.0×10^5 cells/ml. Each 50 µl of this cell suspension was inoculated to each well of a round bottom 96-well plate, and the suspension was cultured in a 5% CO_2 incubator (37 °c) overnight. After culturing overnight, 50 µl of BSA-RPMI1640 containing 200 ng/ml SCF (R&D Co., Ltd.) and 100 µl of BSA-RPMI1640 containing a diluted test substance were added to each well.

[0045] On the 7th day after addition of the test substance, 20 µl of Cell Counting Kit-8 (Dojin Laboratories) was added to the well and was cultured in a 5% CO_2 incubator (37 °c) for about 2 hours. After color development, the absorbance of each well was determined using a MTP-32 plate reader (Colona Electric Co., Ltd.) at a measuring wavelength of 450 nm and at a reference wavelength of 660 nm. The absorbance of each well was subtracted by the absorbance of the well without addition of SCF, and then the ratio of the

absorbance of the well with addition of the test substance to the ratio of the absorbance of the well without addition of the test substance was determined. This ratio was used to calculate the concentration of the test substance required for 50% inhibition of the cell proliferation (IC_{50}).

[0046] Consequently, Compounds 1, 2, 3 and 4 inhibited the cell proliferation stimulated by SCF as shown in the table below, and these compounds were considered to possess c-Kit kinase inhibitory activity. The IC_{50} of the compound KRN633, which is described in Kazuo Kubo et al., 22nd Symposium on Medicinal Chemistry, Abstracts, pp. 275-277, 2P-320, 2002, proved to be 301 nM and the compound showed only weak activity as compared to Compounds 1, 2, 3 and 4. STI571 known as a c-Kit kinase inhibitor showed IC_{50} of 190 nM.

[0047]

[Table 1]

Compound	IC_{50} (nM)
Compound 1	9.36
Compound 2	12.8
Compound 3	214
Compound 4	56.3

[0048] [Example 2] Effect of Compound 1 on c-Kit kinase phosphorylation by SCF stimulation

[0049] Compound 1 was tested for its effect on the phosphorylation of the c-Kit kinase molecule by SCF stimulation in the small cell lung cancer cell line H-

526 expressing c-Kit kinase.

[0050] H-526 cells were cultured in a 5% CO_2 incubator (37 °c) using an RPMI1640 medium containing 10% FCS. After culturing, H-526 cells were washed with PBS three times and were suspended in a BSA-RPMI1640 medium at $5.0x10^5$ cells/ml. Each 1 ml of this cell suspension was inoculated to the well of a 24-well plate and the suspension was cultured in a 5% CO_2 incubator (37 °c) for 6 hours. After 6-hours culturing, 1 ml of BSA-RPMI1640 containing a diluted test substance was added to each well and culturing was carried out in a 5% CO_2 incubator (37 °c) for 1 hour. Additional culturing was then carried out in a 5% CO_2 incubator (37 °c) for 5 minutes after the addition of 10 µl of SCF (10 µg/ml, R&D Corporation). After 5-minutes culturing, the cells were washed with PBS and 100 µl of SDS sample loading buffer was added to the cells to prepare a cell lysate sample. After the sample was heat-treated at 94 °c for 10 minutes, it was cryopreserved at -20 °c.

[0051] The cell lysate sample, 20 µl, was then electrophoresed on a 4-20% gradient polyacrylamide gel (Daiichi Pure Chemicals Co., Ltd.). After electrophoresis, the sample was transferred to a PVDF membrane (Amersham Pharmacia Biotech Inc.) for 3 hours. The transferred membrane was subjected to immunoblot

using a phospho-c-kit (Tyr719) antibody (Cell Signaling Technology Inc.) as a primary antibody and an anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology Inc.) as a secondary antibody. After the membrane was washed, it was developed with a Super Signal (Pierce Biotechnology, Inc.).

[0052] As the results are shown in Fig. 1, c-kit kinase was not phosphorylated (the farthest left lane) in the absence of SCF, and the addition of Compound 1 suppressed the c-Kit kinase phosphorylation that would take place in the presence of SCF in a concentration-dependent manner. The phosphorylation inhibitory activity of STI571, which is known as a c-Kit kinase inhibitor, was approximately one tenth of that of Compound 1.

[0053] [Example 3] Effect of Compound 1 on growth of H-526 tumor transplanted to nude mice

[0054] H-526 cells were cultured in a 5% CO_2 incubator (37 °c) using an RPMI1640 medium containing 10% FCS. After the culture medium was collected, H-526 cells were washed with PBS twice and were suspended in PBS at 5.0×10^7 cells/ml. This cell suspension (0.1 ml) was transplanted to the subcutaneous parts of the right flank of 6-week female Balb/c nu/nu mice (purchased from Charles River Laboratories, Inc.). After transplantation, administration of a test substance was

started at the point the tumor volume reached approximately 150 mm^3, and thus, oral administration was conducted twice daily for a period of 14 days. The test substance was suspended in a 0.5% methylcellulose solution (Wako Pure Chemical Industries Co., Ltd.) so as to give a dose of 0.1 ml/10 g body weight.

[0055] The tumor volume was measured with a caliper twice weekly during the administration period. The long and short diameters of the tumor were measured with a caliper and the tumor volume was calculated according to the equation: 1/2 x long diameter x short diameter x short diameter. Here, the experiment was conducted in a vehicle control group of 10 animals (solvent-administered group) as well as in a test substance administered group of 5 animals.

[0056] As the results are shown in Fig. 2, Compound 1 suppressed the growth of the nude mouse transplanted H-526 tumor in a dose-dependent manner. On the other hand, STI571 known as a c-Kit kinase inhibitor showed little anti-tumor effect when administered even at 160 mg/kg.

[0057] [Example 4] Effect of Compound 1 on c-Kit kinase phosphorylation in H-526 tumor transplanted to nude mice

[0058] 0.1 ml of a H-526 cell suspension prepared at a concentration of 5.0×10^7 cells/ml, was

transplanted to the subcutaneous parts of the right latus of 6-week female Balb/c nu/nu mice (purchased from Charles River Laboratories, Inc.). The animals were then divided into a vehicle control group (solvent-administered group) and a test substance administered group at the point the tumor volume reached 300-1000 mm^3: the test substance was administered to the latter group. The extracted tumor was placed in a cell lysate buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mm $MgCl_2$, 1 mM EDTA, 100 mM NaF, 1 mM PMSF, 10 μg/ml aprotinin, 50 μg/ml leupeptin, 1 μg/ml peptatin A, 1 mM Na_3VO_4, 25 mM β-glycerophosphate, and phosphatase inhibitor cocktail II) and homogenized. After centrifugation, the supernatant was protein quantified, and a 3xSDS sample loading buffer was added to prepare a cell lysate sample. Subsequently, the cell lysate was heat-treated at 94 °c for 10 minutes and cryopreserved at -20 °c.

[0059] The cell lysate sample which was equivalent to 30 μg of protein was electrophoresed on a 4-20% gradient polyacrylamide gel (Daiichi Pure Chemicals Co., Ltd.). After electrophoresis, the sample was transferred to a PVDF membrane (Amersham Pharmacia Biotech Inc.) for 3 hours. In order to assay phosphorylated c-Kit, c-Kit and β-actin, immunoblot was

performed using a phospho-c-kit (Tyr719) antibody (Cell Signaling Technologies, Inc.), an anti c-Kit antibody (Cell Signaling Technologies, Inc.) and an anti β-actin antibody (Sigma) as a primary antibody and an anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technologies, Inc.) as a secondary antibody. After the membrane was washed, it was developed with a Super Signal (Pierce Biotechnology, Inc.).

[0060] As the results are shown in Fig. 3, Compound 1 reduced phosphorylated c-Kit in tumor tissue when administered at 30 or 100 mg/kg, but c-Kit and β-actin remained unchanged. While Compound 1 completely inhibited phosphorylation when administered at 30 or 100 mg/kg, STI571 known as a c-Kit kinase inhibitor partially inhibited phosphorylation when administered even at 160 mg/kg.

[0061] All these result showed Compound 1 inhibits *in vivo* phosphorylation of c-Kit, and it is confirmed that Compound 1 inhibits activity of c-Kit kinase *in vivo* and exhibits anti-tumor activity.

[0062] Production methods of Compound 1 (4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide) (Reference Example 1 to 3), crystals of methanesulfonate of Compound 1 (Reference Example 4 to 9) and formulation of methanesulfonate of Compound 1 (Reference Example 10)

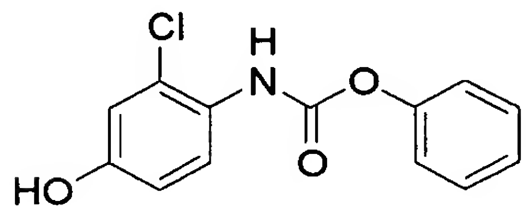
were described below.

[0063] [Reference Example 1] Production method (1) of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide

[0064] To a solution of phenyl N-(4-(6-carbamoyl-7-methoxy-4-quinolyl)oxy-2-chlorophenyl)carbamate (described in WO02/32872; 17.5 g, 37.7 mmol) dissolved in N,N-dimethylformamide (350 mL), was added cyclopropylamine (6.53 mL, 94.25 mmol) under nitrogen atmosphere, and the mixture were stirred at room temperature overnight. The mixture was poured into water (1.75 L), and stirred. Precipitated crude crystals were filtered off, washed with water, and dried at 70 °C for 50 minutes. Ethanol (300 mL) was added to the crude crystals, the mixture was heated to reflux for about 30 minutes to dissolve the crystals, and gradually cooled to room temperature overnight while stirring. Precipitated crystals were filtered off, dried *in vacuo*, and dried at 70 °C for 8 hours to give crystals of the title compound (12.91 g, yield 80.2 %).

[0065] [Reference Example 2] Production method (2) of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide

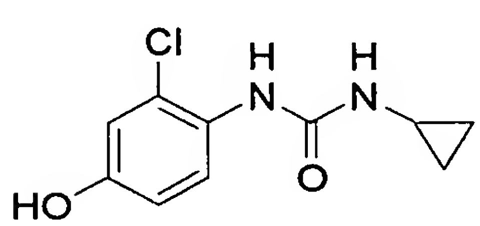
[0066] (1) production of phenyl N-(2-chloro-4-hydroxyphenyl)carbamate



[0067] To a suspension of 4-amino-3-chlorophenol (23.7 g) in N,N-dimethylformamide (100 mL) was added pyridine (23.4 mL) while cooling in an ice-water bath, and phenyl chloroformate (23.2 mL) was added dropwise at a temperature not higher than 20 °C. After the mixture was stirred at room temperature for 30 minutes, water (400 mL), ethyl acetate (300 mL) and 6N-HCl (48 mL) were added thereto, the mixture was further stirred, and the organic layer was separated off. The organic layer was twice washed with 10 % brine (200 mL), and dried over magnesium sulfate. Evaporation of the solvent yielded the title compound (46 g) as a solid.

[0068] ^{1}H-NMR Spectrum ($CDCl_3$) δ(ppm): 5.12 (1H, br s), 6.75 (1H, dd, J=9.2, 2.8 Hz), 6.92 (1H, d, J=2.8 Hz), 7.18-7.28 (4H, m), 7.37-7.43 (2H, m), 7.94 (1H, br s)

[0069] (2) production of phenyl 1-(2-chloro-4-hydroxyphenyl)-3-cyclopropylurea



[0070] To a solution of phenyl N-(2-chloro-4-hydroxyphenyl)carbamate dissolved in N,N-dimethylformamide (100 mL) was added cyclopropylamine (22.7 mL) while cooling in an ice-water bath, and the mixture was stirred overnight at room temperature. Water (400 mL), ethyl acetate (300 mL) and 6N-HCl (55 mL) were added thereto, the mixture was further stirred, and the organic layer was separated off. The organic layer was twice washed with 10 % brine (200 mL), and dried over magnesium sulfate. Evaporation of the solvent yielded prism crystals, the crystals were washed with heptane and filtered off to give the title compound (22.8 g, 77% yield from 4-amino-3-chlorophenol).

[0071] ^{1}H-NMR Spectrum ($CDCl_3$) δ(ppm): 0.72-0.77 (2H, m), 0.87-0.95 (2H, m), 2.60-2.65 (1H, m), 4.89 (1H, br s), 5.60 (1H, br s), 6.71 (1H, dd, J=8.8, 2.8 Hz), 6.88 (1H, d, J=2.8 Hz), 7.24-7.30 (1H, br s), 7.90 (1H, d, J=8.8 H)

[0072] (2) production of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide

[0073] TO dimethylsulfoxide (20 mL) were added 7-methoxy-4-chloroquinoline-6-carboxamide (0.983 g), 1-(2-chloro-4-hydroxyphenyl)-3-cyclopropylurea (1.13 g) and cesium carbonate (2.71 g), and the mixture was

stirred at 70 °C for 23 hours. The mixture was cooled to room temperature, addition of water (50 mL) yielded crystals, and the crystals were filtered off to give the title compound (1.56 g, yield 88%).

[0074] [Reference Example 3] Production method (3) of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide

[0075] To a reaction vessel were added 7-mthoxy-4-chloroinoline-6-carboxamide (5.00 kg, 21.13 mol), dimethylsulfoxide (55.05 kg), 1-(2-chloro-4-hydroxyphenyl)-3-cyclopropylurea (5.75 kg, 25.35 mol) and potassium t-butoxide (2.85 kg, 25.35 mol) under nitrogen atmosphere. The mixture was stirred at 20 °C for 30 minutes, and heated to 65 °C over 2.5 hours. The mixture was stirred at the same temperature for 19 hours, and 33% (v/v) acetone-water (5.0 L) and water (10.0 L) were added dropwise over 3.5 hours. After the addition, the mixture was stirred at 60 °C for 2 hours, and 33% (v/v) acetone-water (20.0 L) and water (40.0 L) were added dropwise at a temperature not lower than 55 °C over 1 hour. The mixture was stirred at 40 °C for 16 hours, precipitated crystals were filtered off by a nitrogen pressured filter and washed with 33% (v/v) acetone-water (33.3 L), water (66.7 L) and acetone (50.0 L). Resultant crystals were dried at

60 °C for 22 hours by a conical vacuum drier to give the title compound (7.78 kg, yield 96.3%).

[0076] The values of ^{1}H-NMR chemical shift of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide obtained in Reference Examples 1 to 3 corresponded to those described in WO02/32872.

[0077] [Reference Example 4] Production method of crystals (A) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0078] (Method 1) 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (700 mg, 1.64 mmol) was dissolved in a mixed solution of methanol (14 mL) and methanesulfonic acid (143 µL, 1.97 mmol) at 70 °C. After the confirmation of the dissolution of the compound, the mixture was cooled to room temperature over 5.5 hours, stirred at room temperature for 18.5 hours, and crystals were filtered off. The resultant crystals were dried at 60 °C to give the title crystals (647 mg).

[0079] (Method 2) 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (600 mg, 1.41 mmol) was dissolved in a mixed solution of acetic acid (6 mL) and

methanesulfonic acid (200 μL, 3.08 mmol) at 50 °C. After the confirmation of the dissolution of the compound, to the mixture were added ethanol (7.2 mL) and seed crystals (A) of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (12 mg), and ethanol (4.8 mL) was added dropwise over 2 hours. After the addition, the mixture was stirred at 40 °C for 1 hour and at room temperature for 9 hours, and crystals were filtered off. The resultant crystals were dried at 60 °C to give the title crystals (545 mg).

[0080] [Reference Example 5] Production method of crystals (B) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0081] Crystals (I) of an acetic acid solvate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (250 mg) prepared in Reference Example 9 were dried under aeration at 30 °C for 3 hours and 40 °C for 16 hours to give the title crystals (240 mg).

[0082] [Reference Example 6] Production method of crystals (C) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0083] (Method 1) n-Butyl acetate (12 mL) was

added to crystals of a dimethylsulfoxide solvate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (600 mg, 1.15 mmol) prepared in Method 1 of Reference Example 7, the mixture was stirred at 115 °C for 10 hours and at room temperature for 1.5 hours, and crystals were filtered off. Drying under aeration at 60 °C gave the title crystals (503 mg).

[0084] (Method 2) Ethanol (6.4 mL) was added to crystals (I) of an acetic acid solvate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (1.28 g) prepared in Reference Example 9, the mixture was dissolved at 40 °C, and stirred at the same temperature for 36 hours. Precipitated crystals were filtered off, and dried at 50 °C to give the title crystals (0.87 g).

[0085] (Method 3) In a mixed solution of acetic acid (14 mL) and methanesulfonic acid (0.37 mL, 5.62 mmol) were dissolved 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (2.00 g, 4.69 mmol) at 40 °C. After the confirmation of the dissolution, to the mixture were added 2-propanol (9 mL) and seed crystals (C) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (100 mg). The

mixture was stirred for 20 minutes, isopropyl acetate (10 mL) was added dropwise over 30 minutes. After the addition of isopropyl acetate, the mixture was stirred for 1.5 hours and at 15 °C for 14 hours. Precipitated crystals were filtered off, and dried at 60 °C to give the title crystals (2.22 g).

[0086] (Method 4) 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (1.28 g, 3 mmol) and acetic acid (12.8 mL) were mixed, methanesulfonic acid (0.408 mL, 6.3 mmol) was added to this suspension, and the mixture was stirred at room temperature for dissolution. The mixture was heated in a bath at a temperature of 30 °C, and 2-propanol was added (7.7 mL). Seed crystals (C) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate was added, and 2-propanol (1.28 mL each, 14 times) was added over 44 minutes. After removal of the bath, the mixture was stirred at room temperature for 10 minutes, stirred in a water bath for 5 minutes, and further stirred in the water bath cooled with a little ice for 25 minutes (inner temperature 17.6 °C). The resultant crystals were filtered off, and washed with 2-propanol (10 mL). The resultant crystals after filtration were mixed with ethanol (6.4 mL) and the mixture was stirred at room

temperature for 1 hour. The resultant crystals were filtered off, washed with ethanol (4 mL), and dried at 60 °C to give the title crystals (1068 mg).

[0087] [Reference Example 7] Production method of crystals of a dimethylsulfoxide solvate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0088] (Method 1) Dimethylsulfoxide (7 mL) was added to 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (700 mg, 1.640 mmol), and the compound was dissolved at 80 °C. To the mixture were added methanesulfonic acid (143 μL, 1.97 mmol), ethyl acetate (1.4 mL) and seed crystals (A) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate at 60 °C, and ethyl acetate (5.6 mL) was further added dropwise over 45 minutes. Fifteen minutes after the completion of the addition of ethyl acetate, the mixture was cooled to room temperature over 1 hour, and stirred at the same temperature for 18 hours. Precipitated crystals were filtered off, and dried at 60 °C to give the title crystals (746 mg).

[0089] (Method 2) Dimethylsulfoxide (6.8 mL) was added to 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-

quinolinecarboxamide (854 mg, 2 mmol) at room temperature, and the compound was dissolved at 60 °C. To the mixture were added methanesulfonic acid (389 µL, 6 mmol) and seed crystals (A) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate at the same temperature, and 2-propanol (6.8 mL) was added dropwise over 30 minutes. After the addition of 2-propanol, the mixture was cooled to 15 °C over 2 hours, and stirred at the same temperature for 30 minutes. Precipitated crystals were filtered off, and dried at 60 °C to give the title crystals (1095 mg).

[0090] (Method 3) Dimethylsulfoxide (6.8 mL) was added to 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (854 mg, 2 mmol) at room temperature, and the compound was dissolved at 62 °C. To the mixture were added methanesulfonic acid (454 µL, 7 mmol) and seed crystals (A) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate at the same temperature, and 2-propanol (13.6 mL) was added dropwise over 1 hour. After the addition of 2-propanol, the mixture was cooled to 15 °C over 2 hours, and stirred at the same temperature for 30 minutes. Precipitated crystals were filtered off, and dried at

60 °C to give the title crystals (1082 mg).

[0091] [Reference Example 8] Production method of crystals (F) of a hydrate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0092] In a mixed solution of acetic acid (1.5 mL) and methanesulfonic acid (31 μL, 0.422 mmol) was dissolved 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (150 mg, 0.351 mmol) at 50 °C. After the confirmation of the dissolution, to the mixture were added ethyl acetate (0.6 mL) and seed crystals (A) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate prepared in Method 1 of Reference Example 4, and ethyl acetate (1.8 mL) was added dropwise over 2 hours. After the addition of ethyl acetate, the mixture was stirred at 50 °C for 30 minutes, and at room temperature for 7.5 hours. Precipitated crystals were filtered off, and dried at 60 °C to give the title crystals (176 mg).

[0093] [Reference Example 9] Production method of crystals (I) of an acetic acid solvate of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0094] In a mixed solution of acetic acid (14 mL)

and methanesulfonic acid (0.36 mL, 5.62 mmol) was dissolved 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide (2.00 g, 4.69 mmol) at 40 °C. After the confirmation of the dissolution, to the mixture were added 1-propanol (4 mL) and seed crystals (C) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate (100 mg), and 1-propanol (14 mL) and isopropyl acetate (10 mL) were added dropwise over 1 hour. After the addition, the mixture was stirred at 40 °C for 1 hour and at 25 °C for 40 minutes. Precipitated crystals were filtered off to give the title crystals (2.61 g).

[0095] ^{1}H-NMR chemical shift of the methanesulfonate is as follow.

[0096] ^{1}H-NMR Spectrum (DMSO-d_6) δ(ppm): 0.44 (2H, m), 0.67 (2H, m), 2.36 (3H, s), 2.59 (1H, m), 4.09 (3H, s), 6.95 (1H, d, J=7 Hz), 7.25 (1H, d, J=2 Hz), 7.36 (1H, dd, J=3, 9 Hz), 7.63 (1H, d, J=3 Hz), 7.65 (1H, s), 7.88 (1H, brs), 7.95 (1H, brs), 8.06 (1H, s), 8.37 (1H, d, J=9 Hz), 8.73 (1H, s), 8.97 (1H, d, J= 7 Hz)

[0097] [Reference Example 10] Formulation of 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

[0098] Based on the prescription described in

Table 2, 0.1 mg tablet was produced by the following method 1, and 1 mg tablet and 10 mg tablet were produced by the following method 2.

[0099] (Method 1) After mixing D-mannitol, crystalline cellulose and hydroxypropylcellulose, a major ingredient dispersed in an appropriate amount of ethanol added to the mixture for granulation. The granulated product was dried and then size-controlled. Sodium croscarmellose and sodium stearyl fumarate were added to the resultant granule and mixed, then were subjected to tableting. The resultant tablets were film coated with a mobile layer using a mixture of coating base.

[0100] (Method 2) After mixing a major ingredient and light anhydrous silicic acid, D-mannitol, crystalline cellulose and hydroxypropylcellulose were further added and mixed. An appropriate amount of ethanol was added for granulation. The granulated product was dried and then size-controlled. Sodium croscarmellose and sodium stearyl fumarate were added to the resultant granule and mixed, then were subjected to tableting. The resultant tablets were film coated with a mobile layer using a mixture of coating base.

[0101]

[Table 2]

Material	Purpose	0.1 mg tablet	1 mg tablet	10 mg tablet
compound*1	major ingredient	0.1	1	10
light anhydrous silicic acid	excipient	0	8	32
D-mannitol	excipients	60.4	51.5	200
crystalline cellulose	excipients	30	30	120
hydroxypropylcellulose	binder	3	3	12
sodium croscarmellose	disintegrator	5	5	20
sodium stearyl fumarate	lubricant	1.5	1.5	6
a mixture of coating base*2	coating agent	5	5	11
total		105	105	411

*1: 4-(3-Chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide methanesulfonate

*2: a pre-mixed material consisting of 56.0% hydroxypropylmethylcellulose, 28.0% talc, 10.0% Macrogol 6000, 4.0% titanium oxide and 2.0% yellow iron sesquioxide (w/w %).

[0102] It was discovered that a compound represented by the general formula (I) shows a strong c-Kit kinase inhibitory activity, and it inhibits proliferation of c-Kit kinase activated-cancer cells both *in vitro* and *in vivo*. Therefore, the compound represented by the general formula (I) is shown to be applicable as an anti-cancer agent for cancers malignant-transformed by activation of c-Kit kinase. Moreover, a c-Kit kinase inhibitor comprising as an active ingredient the compound represented by the general formula (I) is suggest to be effective for diseases such as mastocytosis, allergy and asthma, which are considered to be caused by c-Kit kinase.